REMARKS

Claims 1, 2, 7-18, 20-28, 33-44, and 47-55 were pending prior to this response. By the present communication, claims 22 and 50 have been cancelled without prejudice, no claims have been added, and claims 1 and 26 have been amended to define Applicants' invention with greater particularity. The claim amendments add no new matter, being fully supported by the Specification and original claims. Accordingly, claims 1, 2, 7-18, 20, 21, 23-28, 33-44, 47-49, and 51-55 are currently pending.

The Rejection Under 35 U.S.C. § 102 (e)

Applicants respectfully traverse the rejection of claims 1-4, 6-10, 16-19, 23-27, 29, 30, 32-36, 39-45, and 51-53 under 35 U.S.C. § 102 (e) as allegedly being anticipated by Thompson et al. (U.S. Patent No. 5,824,485; hereinafter "Thompson"). Claims 19, 29, 30, 32 and 45 have been cancelled, thereby rendering the rejection moot as to those claims. Therefore, Applicant will address the rejection as to the currently presented claims.

Applicants respectfully submit that the invention methods for identifying bioactivities or biomolecules, as defined by amended claims 1 and 26, distinguish over the disclosure of the Thompson by requiring, "generating a normalized environmental library". The library contains a plurality of clones in *E.coli,* wherein the nucleic acid for generating the library is naturally occurring and obtained from a mixed population of uncultured organisms.

Applicants describe "normalizing" and the advantages of libraries that are "normalized" in U.S. Patent 6,174,673 (hereinafter "the '673 patent"), which is incorporated by reference into the present application:

> One embodiment for forming a normalized library from an environmental sample begins with the isolation of nucleic acid from the sample. This nucleic acid can then be fractionated prior to normalization to increase the chances of cloning DNA from minor species from the pool of organisms sampled. DNA can be fractionated using a density centrifugation technique, such as a cesium-chloride gradient. When an intercalating agent, such as bis-benzimide is employed to change the buoyant density of the nucleic acid, gradients will fractionate the DNA based on relative base content. Nucleic acid from multiple organisms can be separated in this manner, and this technique can be used to fractionate complex mixtures of genomes. This can be of particular value when working with complex environmental samples. . . This "normalization" approach reduces the redundancy of clones from abundant species and increases the representation of clones from rare species. These normalized libraries allow for greater screening efficiency resulting in the identification of cells encoding novel biological catalysts.

In the '673 patent, Applicants also teach: "single-stranded nucleic acid representing an enrichment of rare sequences is amplified using techniques well known in the art, such as a polymerase chain reaction (Bames, 1994), and used to generate gene libraries. This procedure leads to the amplification of rare or low abundance nucleic acid molecules, which are then used to generate a gene library which can be screened for a desired bioactivity."

Thompson is silent regarding "normalizing" polynucleotides, as the term is used in Applicants' specification and claims, to form a normalized library of DNA clones, which is then screened. Thompson's few comments regarding amplifying the copy numbers of a genomic library occur in two contexts. The first of these in Section 5.3.3. ENRICHMENT OF NON-RIBOSOMAL SEQUENCES FROM TOTAL RNA, concerns, as the title describes, separation of ribosomal sequences from total RNA. In the second, Section 5.4.8. PRE-SCREENING OF EXPRESSION LIBRARIES, three categories of pre-screening are described:" intracellular pre-

screening", which entails introduction of the library into a host engineered to contain a chemo-responsive reporter construct and selecting cells by fluorescence-activated cell sorting (FACS) or macrodroplet sorting; 2) differential pre-screening, which entails incubation of the library in the host with fluorescent or chromogenic physiological tracers, followed by FACS or macrodroplet sorting; and 3) "selection pre-screening," which entails incubation of the library in the host with selective agents such as antibiotics, followed by FACS or macrodroplet sorting to identify surviving or multiplying cells. For all of these methods, cell sorting is done on bulk cultures of amplified libraries prior to examination of individual cultures. Thus, Applicants' goal for the normalization step is preparation of *naturally occurring molecules* for equal representation in a library followed by screening.

By contrast, to reduce the number of clones that need to be screened, Thompson describes pre-selection of DNA fragments for the screening library using probes and refers to this process as "biasing" a library. Such probes are described as being "prepared from known genes that may be related to or are involved in producing compounds of interest" (Thompson, Col 32, lines 6-7). However, rather than using the probes for screening (e.g., identifying molecules having a nucleotide sequence complementary to the probes) of a library of already "normalized" naturally occurring molecules, as in Applicants' claims 1 and 26, Thompson uses the activity probe concept for preparing "chimeric" and "biased" *combinatorial expression libraries*" (See Thompson, Section 5.1.6) prior to screening.

Applicants provide extrinsic evidence in support of the meaning of "combinatorial" as used in Thompson to distinguish such teaching from Applicants' teaching and claims directed to screening of naturally occurring molecules. Exhibit A is a print out from an internet site that includes a description of Neugenesis' combinatorial biology technology, which creates

"combinatorial panels of heavy and light chains of a heteromeric protein and to build libraries of diverse, new, fully assembled proteins. Variants of each subunit gene are generated within the host by Neugenesis' proprietary technology." (http://www.neugenesis.com/) Clearly, Applicants' claims are not directed to combinatorial approaches to identifying enzyme activities encoded by naturally occurring gene clusters, since Applicants are not manipulating the DNA to generate variants.

Exhibit B is a printout from the internet site of the Koide Group, from University of Pittsburgh (http://www.pitt.edu/~sparano/group/). As you will note, the study of Natural Products is separate and distinct from the study of Combinatorial Libraries. Exhibit C provides a glossary of terms used in Medicinal Chemistry. On page 4, the term combinatorial synthesis is described as "...combining sets of building blocks" e.g., ligating together individual genes of a gene cluster.

Thus, in view of Thompson's use of "biased" libraries in the context of preparing combinatorial libraries, Applicants submit that Thompson neither discloses "normalization" of naturally occurring molecules as the term normalization is used in Applicants' claims 1 and 26, nor uses fluorescent probes for screening of such libraries. Accordingly, Applicants respectfully submit that Thompson fails to disclose each and every element of independent claims 1 and 26 (and dependent claims 2, 7-10, 16-18, 23-25, 27, 33-36, 39-44, and 51-53) as would be required to establish anticipation under 35 U.S.C. 102(e).

The Rejection under 35 U.S.C. § 103

To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to

combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all of the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, and not based on applicant's disclosure. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991). The mere fact that references can be combined or modified does not render the resultant combination obvious unless the prior art also suggests the desirability of the combination. *In re Mills*, 916 F.2d 680, 16 USPQ2d 1430 (Fed. Cir. 1990).

Applicants respectfully traverse the rejection of claims 1-4, 6-15, 16-19, 23-27, 29, 30, 32-45 and 51-53 under 35 U.S.C. § 103 as allegedly being unpatentable over Thompson et al. in view of Plovins et al. and Zhang et al. Applicants respectfully submit that the invention methods for high throughput screening of nucleic acid for identifying bioactivities or biomolecules, as recited by amended claims 1 and 26, distinguish over the disclosure of Thompson, at least by reciting:

> generating a normalized environmental library containing a plurality of clones in *E.coli,* wherein the nucleic acid for generating the library is naturally occurring and obtained from a mixed population of uncultured organisms.

Thus, Applicants' invention, as defined by amended claims 1 and 26, is not the purposeful creation of novel activities or pathways by combinatorial techniques, but rather expression cloning of naturally occurring DNA derived from a mixed population of organisms to produce libraries of naturally occurring activities or gene clusters or pathways or genes as found in nature, without manipulation. In addition, polynucleotides in the libraries are equally represented (i.e., "normalized") on some basis selected to assure that those from organisms whose presence in the

sample is rare on the selection basis, can have an equal chance that a naturally occurring "activity" encoded therein will be discovered as do organisms whose populations predominate in the sample. Thus the polynucleotides in Applicants' library are both "normalized" and naturally occurring, meaning that the polynucleotides and have not been rearranged or recombined in a laboratory setting for the purpose of creating new, combinatorially produced, pathways.

The deficiencies of Thompson described above for disclosing the invention methods apply equally and are incorporated here. In addition, Applicants respectfully submit that Thompson fails to suggest the invention methods and would not motivate those of skill in the art to modify Thompson to arrive at the presently presented invention methods because the thrust of Thompson's disclosure is devoted to preparation and screening of combinatorial gene libraries and Thompson's comments regarding preparation of "biased" libraries pertain specifically to the preparation of such combinatorial libraries. Specifically, Thompson's "biasing" technique does not suggest and would not motivate those of skill in the art to reduce the size of a collection of naturally occurring polynucleotides derived from a mixed population of organisms to increase the chances that an activity encoded by a rare organism in the sample will be as likely to be discovered in the screening as that of an organism whose presence predominates in the sample.

Applicants submit that the disclosures of Plovins and Zhang fail to remedy the deficiencies of Thompson under 35 U.S.C. § 103. The Examiner relies upon Plovins as disclosing use of FDG as well as C_{12}FDG as substrates in animal, bacterial and yeast cells. However, like Thompson, Plovins is completely silent regarding screening of a *normalized* library containing a plurality of clones obtained from a mixed population of organisms. Thus, Applicants respectfully submit that the combined disclosures of Thompson and Plovins fail to teach or suggest the invention methods for identifying bioactivities or biomolecules using high

throughput screening, as defined by amended claims 1 and 26. Further, Applicants submit that the combined disclosures of Thompson and Plovins would be insufficient to motivate those of skill in the art to create a method for screening naturally occurring polynucleotides in which the chances of discovering a desired activity encoded by a rare organism in the sample are normalized with those of species in the sample that predominate.

Like Plovins, Zhang also fails to cure the deficiencies of Thompson for teaching or suggesting the invention methods for identifying bioactivities or biomolecules using high throughput screening, as defined by amended claims 1 and 26. The Examiner relies upon Zhang for disclosure of the development of lipophilic, fluorogenic substrates derived from FDG, such as FDG having an added lipophilic tail, to enable the substrate to pass through the cellular membrane. However, Zhang fails to disclose screening of a *normalized* library containing a plurality of clones obtained from a mixed population of organisms. Thus, Applicants respectfully submit that the combined disclosures of Thompson and Zhang fail to teach or suggest the invention methods for identifying bioactivities or biomolecules using high throughput screening, as defined by amended claims 1 and 26. Further, Applicants submit that the combined disclosures of Thompson and Zhang would be insufficient to motivate those of skill in the art to create a method for screening naturally occurring polynucleotides in which the chances of discovering a desired activity encoded by a rare organism in the sample are normalized with those of species in the sample that predominate.

In view of the failure of either Plovins or Zhang to cure the above-described deficiencies of Thompson for suggesting the invention methods, Applicants respectfully submit that the combined disclosures of Thompson, Plovins and Zhang are not sufficient to teach or suggest the present invention, as defined by amended claims 1 and 26. In addition, even if those of skill in

the art were motivated by the combined disclosures of Thompson, Plovins and Zhang to arrive at the invention methods, Applicants submit that the cited art would fail to provide the reasonable expectation of success that is required to show unpatentability under 35 U.S.C. § 103. Because neither Thompson, Plovins, nor Zhang discuss any technique by which a diverse library can be adjusted to provide equal representation of the polynucleotides obtained from rare members, those of skill in the art would not be justified in assuming success in the outcome of any technique that might be devised.

Thus, Applicants respectfully submit that claims 1-4, 6-15, 16-19, 23-27, 29, 30, 32-45 and 51-53 are not *prima facie* obvious over Thompson, or the combined disclosures of Thompson, Plovins and Zhang. Accordingly, reconsideration and withdrawal of the rejection under 35 U.S.C. § 103 are respectfully requested.

CONCLUSION

In view of the above amendments and remarks, reconsideration and favorable action on claims 1, 2, 7-18, 20, 21, 23-28, 33-44, 47-49, and 51-55 are respectfully requested. If the Examiner would like to discuss any of the issues raised in the Office Action, Applicant's representative, can be reached at (858) 526-5176.

Respectfully submitted,

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COMBINATORIAL BIOLOGY TECHNOLOGY

Neugenesis' combinatorial biology technology (the CombiKARYON™ system) mimics the immune system's ability to generate diversity in antibodies, and expands the application to all heteromeric proteins. The company uses this technology to discover and improve complex proteins. It is an efficient and cost-effective method to improve a protein's stability, affinity, receptor binding capacity, and therapeutic efficacy, thereby enhancing the value of the protein, and decreasing the risk of clinical failure.

Using Combinatorial Biology to Generate Diversity

An outgrowth of Neugenesis' protein production systems, CombiKARYON™ uses the unique features of the filamentous fungus, *Neurospora crassa*, to create combinatorial panels of heavy and light chains of a heteromeric protein and to build libraries of diverse, new, fully assembled proteins. Variants of each subunit gene are generated within the host by Neugenesis' proprietary technology. Strains carrying these new gene sequences are fused to one another in all possible combinations to produce libraries in the following manner.

VARIANT	Light chain 1	Light chain 2	Light chain 3	Light chain 4
Heavy chain 1	L1H1	L2H1	L3H1	L4H1
Heavy chain 2	L1H2	L2H2	L3H2	L4H2
Heavy chain 3	L1H3	L2H3	L3H3	L4H3
Heavy chain 4	L1H4	L2H4	L3H4	L4H4

In this illustration, 16 unique monoclonal antibody combinations are produced from 4 light and 4 heavy chain subunit variants. In a standard microtiter plate configuration, 96 unique combinations would be produced when 12 variants of one subunit are arrayed against 8 variants of the second subunit. With CombiKARYON™, this would be done with 20 total transformations. Traditional protein engineering techniques would require 96 transformations after a complicated reassembly process of the subunit genes. These burdensome steps are eliminated using Neugenesis' combinatorial biology approach. The advantages become more apparent in larger libraries. For example, a 100x100

matrix to create 10,000 combinations would require 200 transformations in the CombiKARYON™ system, and 10,000 transformations using traditional techniques. This technology can also be used to create combinations of more than two subunits, to geometrically increase the diversity. The last step is to screen the combinatorial libraries for new proteins with the desired characteristics.

Applying Combinatorial Biology to Drug Discovery and Improvement

CombiKARYON™ is an expedient approach for companies involved in developing difficult and complex protein therapeutics. The applications of the technology are numerous. For example, Neugenesis' technology may be applied to protein hits to improve the characteristics such as binding capacity or stability. By designing and creating small changes in the original molecule, Neugenesis is able to fine-tune the protein without dramatically changing the protein's core structure, which has already been selected for through years of evolution. This technology can also be applied to protein drug candidates already in pre-clinical and clinical trials. The failure rate of drug candidates in the development process is estimated to be at least 60-80%. Neugenesis' combinatorial biology system may help by providing a means to more efficiently fine-tune these candidates into better, therapeutically useful molecules.

Other potential applications of CombiKARYON™ include drug combination research and hybridization. In drug combination research, combinatorial biology enables rapid and inexpensive creation of any number of combinations of synergistic proteins, which can then be screened for the most effective combination. In hybridization, combinatorial biology can be used to develop hybrid molecules with both binding and effector moieties, improving the specificity of therapeutic agents.

In addition, cultures of desirable molecules identified through this technology can be easily expanded to produce large-scale quantities of the new heteromeric protein for further evaluation, since the protein is already in a *Neurospora* production strain.

Protein Expression | Neugenesis' Homepage | Company Overview | Neugenesis in the News | Technology Tour

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Glossary of Terms Used in Medicinal Chemistry
(IUPAC Recommendations 1998)

A to H

Contents

Active transport*

Active transport is the carriage of a solute across a biological membrane from low to high concentration that requires the expenditure of (metabolic) energy.

Address-message concept

Address-message concept refers to compounds in which part of the molecule is required for binding (address) and part for the biological action (message).

ADME

Abbreviation for **Absorption, Distribution, Metabolism, Excretion.** (See also **Pharmacokinetics; Drug disposition).**

Affinity

Affinity is the tendency of a molecule to associate with another. The **affinity** of a **drug** is its ability to bind to its biological target (**receptor, enzyme**, transport system, etc.) For pharmacological **receptors** it can be thought of as the frequency with which the **drug**, when brought into the proximity of a **recept r** by diffusion, will reside at a position of minimum free energy within the force field of that **receptor.**

For an **agonist** (or for an **antagonist**) the numerical representation of **affinity** is the reciprocal of the equilibrium dissociation constant of the ligand-**receptor** complex denoted K_A, calculated as the rate constant for offset (k_{-1}) divided by the rate constant for onset (k_1).

Agonist***

Catabolism consists of reactions involving endogenous organic substrates to provide chemically available energy (e.g., ATP) and/or to generate metabolic intermediates used in subsequent anabolic reactions.

Catabolite

A **catabolite** is a naturally occurring **metabolite**.

Clone*

A **clone** is a population of genetically identical cells produced from a common ancestor. Sometimes, "**clone**" is also used for a number of recombinant DNA (deoxyribonucleic acid) molecules all carrying the same inserted sequence.

Codon*

A **codon** is the sequence of three consecutive **nucleotides** that occurs in mRNA which directs the incorporation of a specific amino acid into a protein or represents the starting or termination signals of protein synthesis.

Coenzyme

A **coenzyme** is a dissociable, low-molecular weight, non-proteinaceous organic compound (often **nucleotide**) participating in enzymatic reactions as acceptor or donor of chemical groups or electrons.

Combinatorial synthesis

Combinatorial synthesis is a process to prepare large sets of organic compounds by combining sets of building blocks.

Combinatorial library

A **combinatorial library** is a set of compounds prepared by combinatorial synthesis.

CoMFA

See **Comparative Molecular Field Analysis**.

Comparative Molecular Field Analysis (CoMFA)**

Comparative molecular field analysis (CoMFA) is a **3D-QSAR** method that uses statistical correlation techniques for the analysis of the quantitative relationship between the biological activity of a set of compounds with a specified alignment, and their three-dimensional electronic and steric properties. Other properties such as hydrophobicity and hydrogen bonding can also be incorporated into the analysis. (See also **Three-dimensional Quantitative Structure-Activity Relationship** [3D-QSAR]).

Computational chemistry**

Computational chemistry is a discipline using mathematical methods for the calculation of molecular